Scientific Electronic Archives
Issue ID: Sci. Elec. Arch. Vol. 11 (5)
October 2018
Article link
http://www.seasinop.com.br/revista/index.php?journal=SEA&page=article&op=view&path%5B%5D=739&path%5B%5D=pdf
Included in DOAJ, AGRIS, Latindex, Journal TOCs, CORE, Discoursio Open Science, Science Gate, GFAR, CIARDRING, Academic Journals Database and NTHRYS Technologies, Portal de Periódicos CAPES.

ISSN 2316-9281

Androecium development of *Oxypetalum appendiculatum* Mart. (Apocynaceae): a taxonomic approach

F. A. Z. Vital[1] & A. T. Nakamura[2]

[1] Universidade Federal de Lavras
[2] Universidade Federal de Uberlândia

Author for correspondence: flavio.zagotta@hotmail.com

Abstract: Circa 80% of the neotropical species of Asclepiadoideae (Apocynaceae) belong to the "MOOG Clade", whose interrelations are poorly established. This study describes the floral morphology, anther development, microsporogenesis, microgametogenesis, and pollinarium and pollen grain morphology of *Oxypetalum appendiculatum* to foster future taxonomic work. In addition to typical morphological features of Asclepiadoideae, as a bifid vinaceous appendix and a translator with a laterally expanded retinaculum, flowers present a clear division of stamens into three groups, with respect to size and arrangement in the flower, which has not yet been reported in the literature. Stamens present connate, subsessile filaments, bisporangiate anthers with six parietal layers, and dicotyledonous development. Endothecium is unthickened; secretory tapetum is biseriate on the adaxial side and multiseriate on the abaxial surface. Successive microsporogenesis produces linear tetrads. Single pollen grains, united in pollinia, are tricellular and inaperturate. Features as bisporangiate anthers and successive microsporogenesis delimit the subfamily. Unthickened endothecium may indicate a derived position of *O. appendiculatum* in the "MOOG Clade".
Keywords: Anther. Endothecium. Microsporogenesis. "MOOG Clade". Subsessile Filament.

Introduction

With circa 355 genera and 3700 to 5100 species, Apocynaceae (Gentianales) occur on all the continents except Antarctica (RAPINI, 2012). Their specimens are easily recognized by the presence of latex and a style head originating from the fusion of the two carpels at style apex (RAPINI, 2012; ENDRESS & BRUYNS, 2000). Asclepiadoideae, a derived subfamily, presents a series of androecium and gynoecium features, associated with a mechanism that increases pollination efficiency, which have evolved in close relation to the fusion of floral organs (ENDRESS, 2016). Among these innovations is the presence of pollinia, which significantly reduces pollen loss during pollination (HARDER & ROUTLEY, 2006; HARDER & JOHNSON, 2008). In addition, the precise location of the floral whorls allowed a fusion resulting in a gynostegium, which both prevents self-pollination and creates specific sites that guide pollinators during pollinarium removal (HARDER & AIZEN, 2010; VIEIRA & SHEPHERD, 2002).

Four lineages of Asclepiadoideae grow in the New World: (1) *Marsdenia* (Marsdenieae); (2) *Cynanchum* subgen. *Mellichampia* (A. Gray ex S. Wats.) Sundell (Cynanchinae); (3) *Asclepias* (Asclepiadinae); and (4) a clade named "MOOG", comprising subtribes Metastelmatinae Endl. ex Meissn., Orthosiinae Liede & Rapini, Oxypetalinae K. Schum., and Gonolobinae (G. Don) Liede (LIEDE-SCHUMANN ET AL., , 2005; RAPINI ET AL., , 2003). Third largest subtribe in the "MOOG Clade", Oxypetalinae underwent generic rearrangements marked by the inclusion of various small genera into *Oxypetalum* and *Philibertia* to avoid a paraphyletic classification of this group (RAPINI ET AL., 2011; LIEDE-SCHUMANN ET AL., 2014). With circa 130 neotropical species (SILVA et al., 2008), *Oxypetalum* R. Br. is the largest genus of Oxypetalinae. Forty-four species, i.e. 47.3% of the 93 species known in Brazil, grow in the state of Minas Gerais (SILVA et al., 2008; VIEIRA & SHEPHERD, 2002).

Since they are used to define subfamilies (CIVEYREL et al., 1998; ENDRESS & BRUYNS, 2000; VERHOEVEN & VENTER, 1998) and tribes (VERHOEVEN & VENTER, 1998; ENDRESS et al., 2014), androecium features are taxonomically important in Apocynaceae. Though still little explored, pollinarium features, mainly with regard to sporangium formation and organization, should also be quite useful for that matter.

The androecium of *Oxypetalum* was studied and made remarkable contributions to the embryological studies of Apocynaceae addressing mainly microsporogenesis and microgametogenesis (CORRY, 1882; FYRE & BLODGETT, 1905; GUIGNARD, 1917; MEYER, 1938; RAU, 1940).

Based on such studies, Maheshwari (1970) reported the plasticity of mother cell division in microspores and characterized the family as heterogeneous since different species of a given genus can present simultaneous or successive cytokinesis. Therefore, embryology studies can be an important tool to elucidate kinship relationships and foster the family taxonomy.

This work thus analyzes the floral morphology of *Oxypetalum appendiculatum*, to describe androecium development, anatomical aspects of anther development, microsporogenesis, and microgametogenesis, in addition to pollinarium and pollen grain morphology. Such information can help understand how the reproductive structures of Apocynaceae have developed and provide resources for the family systematics.

Methods

Botanical materials of *Oxypetalum appendiculatum* were collected in a rocky outcrop (*campo rupestre*) within the boundaries of the town of Lavras, Minas Gerais. Herborized specimens were deposited at the ESAL Herbarium, Federal University of Lavras (voucher number ESAL 27.511).

Buds and flowers at pre-anthesis, anthesis, and post-anthesis were collected to analyze histologically anther walls, microsporogenesis, and microgametogenesis. Material was fixed in Karnovsky's solution for 24 hours (Karnovsky 1965), gradually dehydrated in an ethanol series (10%, 20%, 30%, 40%, 50%, and 60%) and conserved in ethanol 70%.

Samples were dehydrated in a growing ethanol series, infiltrated, and embedded in hydroxyethyl methacrylate (Leica®) to make up permanent slides. Circa 5 µm cross and longitudinal sections were cut using a rotary microtome, stained with Toluidine Blue O (pH 4.7) (O'BRIEN et al., 1964), and mounted in Acrilex 500 ® colorless glass varnish (PAIVA et al., 2006). All the slides were analyzed with optical and stereo microscopes and images were captured with a photomicroscope or a trinocular photo stereomicroscope.

For scanning electron microscope examination, pollinaria of flowers at anthesis were removed, fixed in Karnovsky's solution with 0.05 M sodium cacodylate buffer (pH 7.2) (KARNOVSKY, 1965). Samples were washed in 0.05M sodium cacodylate buffer and post-fixed in osmium tetroxide 1%, between 1-4 hours, at room temperature. They were dehydrated in acetone solution, submitted to CO_2 critical drying point using a BAL-TEC, CPD-030, fixed on a metallic support with silver cement, and coated with gold (10 nm) using a BAL-TEC SCD-050 device. Prepared material was observed and electromicrographed with a LEO-EVO 40 XVP scanning electron microscope.

Results

Anther Wall

Oxypetalum appendiculatum Mart. presents five bithecal, bisporangiate anthers (Fig. 1 - except k) in convex position on the gynostegium (Fig. 1a). Anthers can be classified into three groups, according to their position in the flower bud: (1) two big inferior anthers; (2) two medium-sized intermediate anthers; and (3) a small superior anther (Fig. 1a).

Anther wall development conforms to the dicotyledonous type. Although anthers present different sizes, their wall development, microsporogenesis, and microgametogenesis follow the same pattern. Yet anthers and microsporangia of the same anther do not develop concomitantly (Fig. 1a – 3c).

Locule formation begins in the two basal anthers, where the contiguous locules develop first to form a pollinarium. Then, the adjacent locules of basal and median anthers form simultaneously. Finally, the adjacent locules of the intermediate anthers and of the superior anther complete the development. Longitudinal sections show that locules are located on the inferior half of anthers (Fig. 3d) and that their height varies according to anther size (Fig. 1a).

Anatomically, the anther primordium of O. appendiculatum presents uniseriate protoderm with cuboid cells containing evident nuclei. The ground meristem of anthers divides into two parts: one with cells presenting a dense aspect, which will form a microsporangium, the other, with bigger cells showing a hyaline aspect, will constitute an anther (Fig. 1b).

At the second stage of bud development, a radially elongated primary parietal layer appears in the locule region (Fig. 1c). It undergoes a periclinal division (Fig. 2d) to originate the inner and outer primary parietal layers (Fig. 1d - e). The layer subjacent to the primary parietal layer also elongates radially and undergoes a periclinal division to originate the sporogenous cells, externally, and more meristematic cells, internally (Fig. 1c - d).

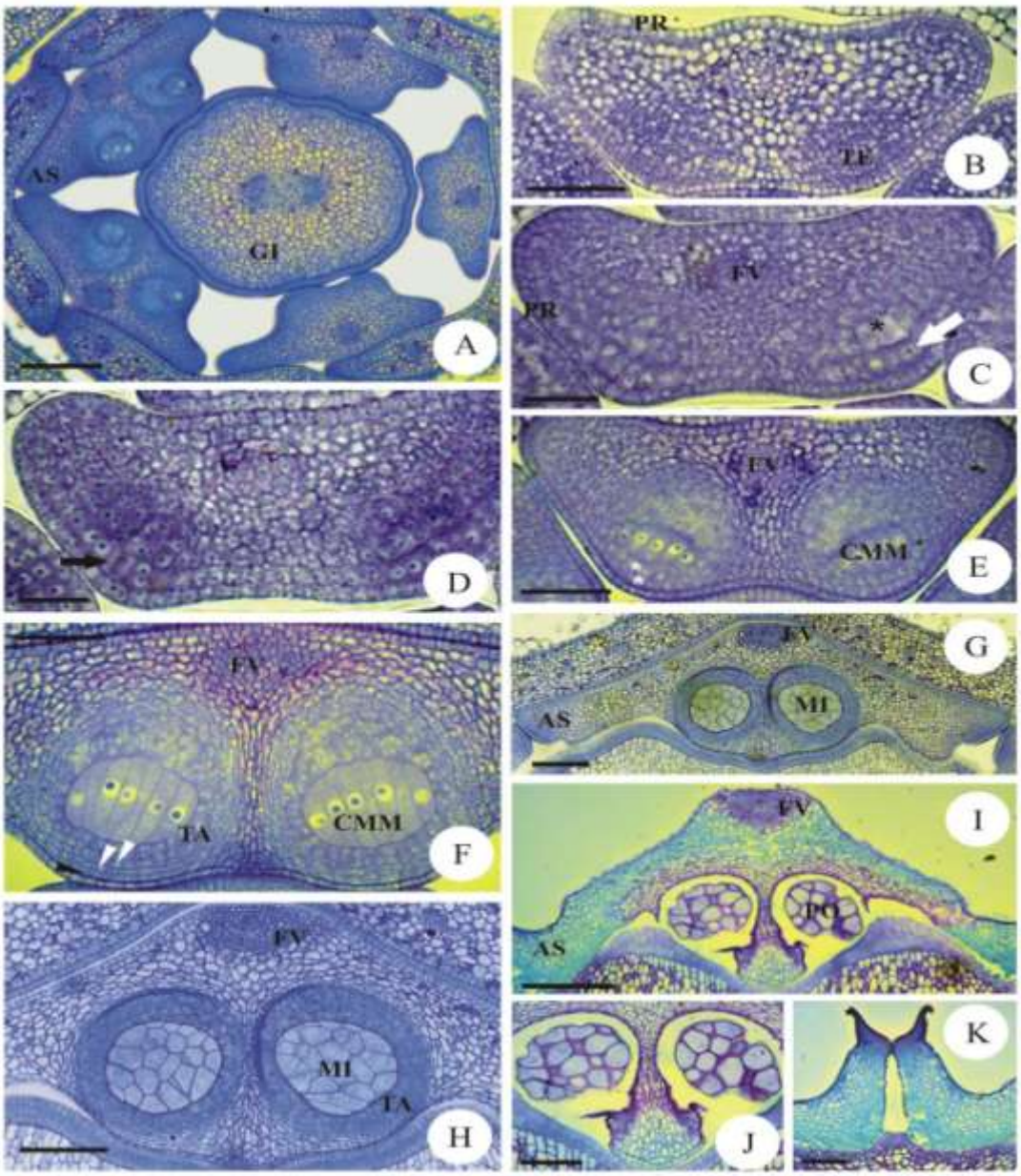

Figure 1. Longitudinal sections of Oxypetalum appendiculatum buds and flowers. A: General aspect of androecium. Left to right, two big inferior anthers, two medium-sized intermediate anthers and a small superior anther. B: Anther primordium showing meristematic tissue. C: Anther primordium showing protoderm, primary parietal layers (WHITE ARROW) and sporogenous cells during mitosis (*). D: Division of primary parietal layers (BLACK ARROW). E: Anther primordium with inner and outer primary parietal layers (*). F: Anther with six parietal layers: epidermis, unthickened endothecium (BLACK ARROWHEAD), two middle parenchymatous layers (WHITE ARROWHEAD), and two strata of tapetum. G: Anther at pre-anthesis, and immature anther wings. H: multiseriate tapetum (*). I: Mature anther with wall lignify and pollinium already isolated in locule. To note stomium break. J: Anther opening. K: Enconter of two adjacent anther wings forming guide rail. AS = anthers wings; CMM = microspore mother cells; FV = vascular bundle; GI = gynoecium; MI = microspore; PC = procambium; PO = pollinium; PR = protoderm; TA = tapetum; TM = meristematic tissue. SCALE BARS: 200 µm (I); 125 µm (A, G); 100 µm (B, J, K); 50 µm (C, D, E, F, H).

The outer primary parietal layer divides to originate the outer secondary parietal layer, which divides again to form the endothecium and the medial layer. The inner secondary parietal layer does not undergo mitotic division and differentiates directly into the tapetum. The meristematic cells of the inner region of the anther locule (anther abaxial surface) divides periclinally and anticlinally to form a tissue

whose cells present thin walls, dense content, and a conspicuous nucleus (Fig. 1e).

Later, the medial layer and the tapetum divide periclinally to forms two layers each (Fig. 1f). Therefore, at this stage, the anther presents six parietal layers: epidermis, unthickened endothecium, two middle parenchymatous layers, and two strata of tapetum. Anther wall formation follows the dicotyledonous type.

It is worth noting that, on the anther abaxial surface, the cells adjacent to the locule, which began differentiating at the previous stage, form a tapetum (Fig. 1f) that is biseriate on the locule adaxial face and multiseriate on its abaxial surface, which is oriented towards the vascular bundle. The former presents cuboid cells with well-defined walls and a conspicuous nucleus located in the central region (Fig. 1f), while the later has radially elongated to cuboid cells. The tapetum of O. appendiculatum is secretory (Fig. 1f-g).

At pre-anthesis, the uniseriate epidermis presents cuboid to slightly convex cells, whose size varies (Fig. 1g-h). The lateral extremities of anther develop a protruding border, the “anther wing”, which gives it its characteristic triangular shape (Fig. 1g). In the distal region of wing, although epidermis elongates radially, it remains single-layered with thin-walled cells and is covered with a thin cuticle (Fig. 1g). Epidermis, endothecium, and middle layers become radially thinner and are compressed in the stomium region. Some tapetum cells can become binucleate (Fig. 1g).

At the stage corresponding to the maximum degree of flower maturity, when anthesis occurs, epidermis, endothecium, and middle layers are obliterated in the stomium region, which turns towards the inner part and contacts the gynostegium (Fig. 1i). Tapetum has already begun to degenerate (Fig. 1i-j). Anther wings grow longer and anther parenchyma, which shows secondary wall deposition, lignifies. On the adaxial face of anther, in the region between thecae, the epidermis and part of the septum also lignify (Fig. 1j). The rest of the epidermis, the vascular bundle, and the region surrounding the locule, with circa six cell layer, that includes part of the septum separating the thecae, do not lignify (Fig. 1i-j). The distal part of anther wings shows the formation of the guide rails (Fig. 1k).

Microsporogenesis and Microgametogenesis

Microspore development begins in the anther primordium when the protoderm involves the meristematic tissue into a differentiation that forms two anther microsporangia. Both are separated by the vacuolated cells constituting the septum, clearly showing the sporangium initial cells, which are isodiametric and present dense cytoplasm (Fig. 2a). Microsporogenesis and microgametogenesis do not occur concomitantly in all the anthers or in the microsporangia of one anther (Fig. 2c).

The archesporial cells begin differentiating when four cells below the primary parietal layer stretch and divide periclinally to form, internally, the meristematic cells that will constitute the multiseriate tapetum (Fig. 1). The four sporogenous cells develop into four cell columns that undergo transversal mitoses to fill the lower half of the anther locule with microspore mother cells arranged in an inclined manner. They become more evident than the other microsporangium cells (Fig. 2d) and undergo a radial mitosis to form eight microspore mother cells, also called microsporocytes (Fig. 2c-e-f).

Each microspore mother cell undergoes a meiosis to form a microspore dyad and, immediately after, a microspore tetrad with walls developing after each division (Fig. 2g), characterizing a successive microsporogenesis. These microspore tetrads are linear (Fig. 2h). At the end of this stage, cytokinesis and callose deposition, which separates the microspores, take place (Fig. 2h) and sporoderm deposition has already begun.

Microgametogenesis then begins and cells undergo asymmetric mitosis to originate bicellular pollen grains (Fig. 2i). The vegetative cell with its central spherical nucleus is located in the central region, while the generative cell is found around it (Fig. 2i). The former, which occupies most of the young gametophyte, undergoes a division that originates two spermatic cells (Fig. 3k). Mature pollen grain is tricellular and inaperturate (Fig. 2j, k, l, m). Sporoderm is smooth and does not present any exine ornamentation (Fig. 2l-m).

In this species, pollen grains are arranged individually in compartments (Fig. 2j-m), but agglutinated in pollinia (Fig. 2k, l, m; Fig. 3 a-d), and enclosed in an amorphous (Fig. 2l-m) and hyaline (Fig. 2k) layer.

Pollinarium

The secretory epidermis of the style head secretes the translator, which comprises a retinaculum and caudicles (Fig. 4a). This fluid secretion deposits in the slits created by the junction of adjacent anther wings (Fig. 4a, f). When anthesis occurs, this fluid contacts a pollinium nestled within an anther locule to form a pollinarium, that is, a set composed by a pollinium (reproductive portion) and a translator (sterile portion) (Fig. 4a-d).

The caudicles, segments of the translator that are in direct contact with the pollinia, presents expansions at apex (Fig. 4a). They show visible damages when a pollinarium is removed from a flower, revealing that the translator is firmly attached to the outer epidermis of anther (Fig 4b, c).

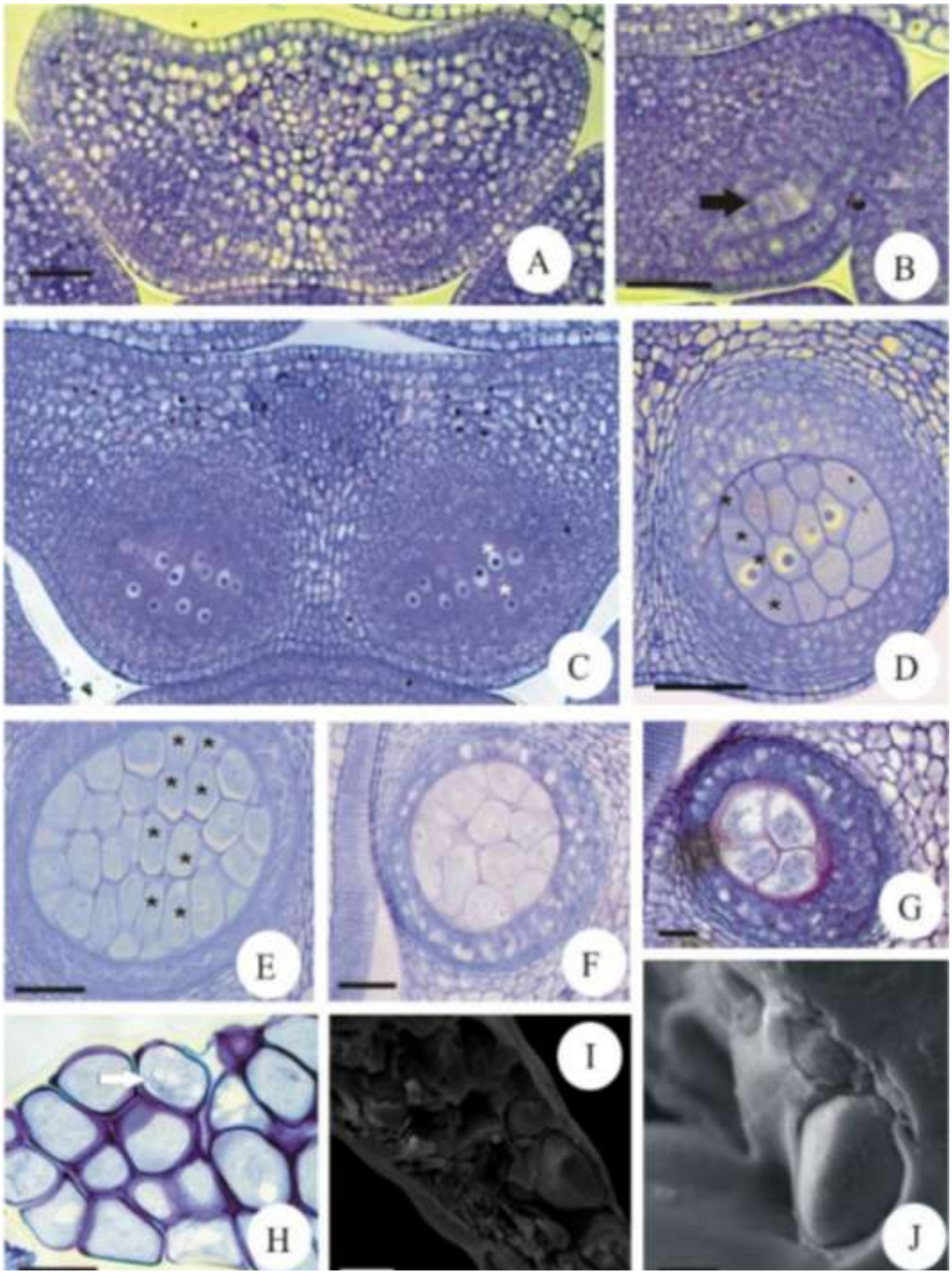

Figure 2. Oxypetalum appendiculatum microsporogenesis and microgametogenesis Anther cross sections, except F, K and L, that are longitudinal sections. I-J: Scanning electron microscope. A: Anther primordium showing protoderm and meristematic tissue. B: sporogenous cells during mitosis (BLACK ARROW). C: Overview of buds showing anthers in diferent height. D: Differentiated sporogenous cells. E, F: Microspores. G: Dyad (*); anther wall after division (BLACK ARROWHEAD). H: Late tetrads (*). I: Bicellular pollen grains. J: Mature pollen grains arranged individually in compartments. K: Pollinarium showing tricellular pollen grain; WHITE ARROW indicate two spermatic cells. L: Overview of pollinium showing each pollen grain at compartments. M: Inaperturate pollen grain. SCALE BARS: 200 µm (C); 100 µm (F); 50 µm (A, B, D, E, H, I, K); 25 µm (G); 20 µm (L); 10 µm (M).

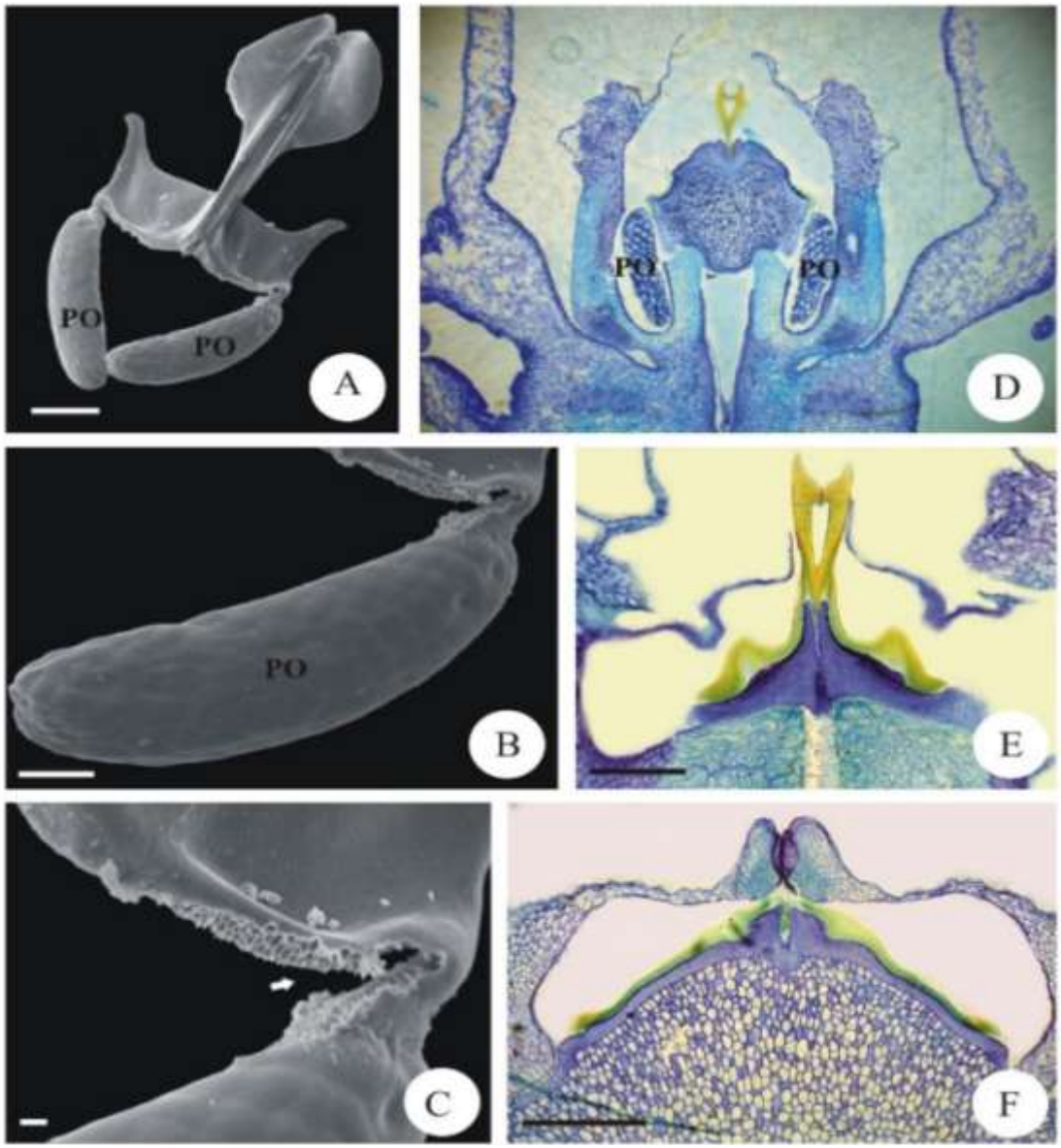

Figure 3. *Oxypetalum appendiculatum* pollinarium. A-C: Scanning electron microscope. Longitudinal sections (D) and cross section (E-F). A: Overview of pollinarium. B. Pollinaria showing the pollen grains at individually compartments. C: Damaged caused when a pollinarium is removed from anther (WHITE ARROW). D: Overview of pollinarium tied to anther. E: Translator, with retinaculum showing "clipping mechanism" and two caudicles in basal part. F: Projection on the gynostegium epidermis fixes the translator. AN = adjacent anther; AS = anther wings; C = caudicles; FI = filament; PO = pollinaria; RE = retinaculum; BLACK ARROW = denticle. SCALE BARS: 250 µm (F); 200 µm (A, D, E); 100 µm (B); 20 µm (C).

The translator presents a winged expansion at retinaculum apex (Fig. 4a) that reaches the apical and adaxial regions. This projection on both sides creates two hooks facing opposite directions that are responsible for the fixation on the pollinating agent, allowing the dispersal of the whole pollinarium (Fig. 4a, d, e). At retinaculum apex, the anther denticles are an active part of this "clipping mechanism" morphology (Fig. 4e). At the basal part of retinaculum, a projection on the gynostegium epidermis fixes the translator and, consequently, the pollinarium (Fig. 4e, f).

The pollinium, in turn, is involved in a film (Fig. 4 a-c). External observation clearly shows the depressions delimiting the chambers in which each pollen grain nestles (Fig. 4a-b). When viewed through an optical microscope, this film is crystalline (Fig. 3h). It involves each pollen grain in individual compartments and the whole pollinium.

Discussion

The bilocular anther of *Oxypetalum appendiculatum* develops according to the dicotyledonous type. It is constituted by six parietal

layers: epidermis, endothecium, two middle layers, and a biseriate secretory tapetum, resembling the description of *Oxypetalum banskii* Roem. et Schult. subsp. *banskii* (VALENTE, 1977). Bilocular anthers are inherent and exclusive to the individuals of Asclepiadoideae. Since the other subfamilies of Apocynaceae present tetralocular anthers (ENDRESS & BRUYNS, 2000), except *Trachelospermum fragrans* (Apocynoideae), which is also bisporangiate (Sud 1984), they indicate a synapomorphic character state.

When they analyzed the pattern of anther development of *Rauvolfia serpentina* (L.) Benth. ex Kurz, Ghimire, Ghimire & Heo (2011) concluded that this species also presents six parietal layers, secretory tapetum, and anther development of the dicotyledonous type. Thus, the anthers of Rauvolfioideae Kostel (*Rauvolfia* L.) and Asclepiadoiedae R. Br. ex Burnett (*Oxypetalum* R. Br.) are anatomically similar, although these subfamilies are located at opposite extremes of the evolutionary derivation of Apocynaceae.

In addition, the anthers of *Vallaris foetida (*L.) Raf., another species of Rauvolfioideae with dicotyledonous development, present 10 to 14 parietal layers (MAHESHWARI, 1970). Therefore, the anther development of the dicotyledonous type can be considered a constant in family Apocynaceae, although the number of parietal layers may vary among members. The number of parietal layers may represent a constant character among species of a given genus, as observed in *Oxypetalum*, but it seems to have evolved more than once, independently.

Manning (1996), who also addressed parietal layers, analyzed 125 families of angiosperms and verified that their endothecium walls present a characteristic thickening. In family Apocynaceae, the endothecium is constituted by elongated cells whose walls present lignified secondary thickening in the form of trabeculae, a character that is not constant in all the subfamilies (KOTOVSKI, 2013; SIMÕES et al., 2006). Kotovski (2013) used the pattern of endothecium lignification of *Allamanda* L. as a taxonomic tool, justifying that the homogenous lignification of endothecium is a feature that maintains this genus in Rauvolfioideae.

However, *O. appendiculatum* shows no evidence of thickening, since its endothecium exhibits little apparent parenchymatous cells in the anther. Anther lignification, nevertheless, is massive, as a whole, and the distal ends of wings exhibit a guide rail. Simões et al., (2006) reported that the formation of guide rails directing the pollinator's mouth apparatus and legs is also found in the endothecial thickening of tribe Mesechiteae Miers. (Apocynoideae).

Rauvolfioideae (except genera of Tabernaemontaneae G. Don) and Periplocoideae R. Br. ex Endl do not show specialized anther lignification, whereas the other subfamilies present lignification occurring in different anther regions, as apex or base or even along its whole extension (ENDRESS & BRUYNS, 2000). Valente & Costa (2005) showed the presence of endothecial bars of thickening subjacent to the locules in the anthers of *Marsdenia loniceroides* E. Fournier.

Phylogenetically, *M. loniceroides* is inserted in tribe Marsdenieae, a tribe with endothecial thickening that is not part of the "MOOG Clade", while *O. appendiculatum*, inserted in tribe Asclepiadeae, within the "MOOG Clade" (LIEDE-SCHUMANN et al., 2005), does not present endothecial thickening. This leads to conclude that endothecial thickening can be a character inherent only to ancestral tribes of this clade. It is suggested that the absence of endothecial thickening in *O. appendiculatum* is justified by the fact that the function of assisting anther opening is perfectly performed by the lignified tissues of anthers. In addition, the secondary thickening of the anther walls, which imprisons the pollinator, helps the pollination process (KOTOVSKI, 2013).

The secretory tapetum, which is biseriate on the adaxial face and multiseriate on the abaxial face of the studied species, plays many functions, among which microspore nutrition and pollen grain development, production of locular fluid, callase, polysaccharides, exine precursors, viscin ligaments, orbicules, proteins, enzymes, and "pollenkitt"/tryphine (DEMARCO, 2015; PACINI, 2010). A secretory tapetum with voluminous anthers and many microspores/pollen grains is a feature inherent to the species of Apocynaceae (DEMARCO, 2015; PACINI et al., 1985).

Biseriate tapetum has been reported in species of subfamily Asclepiadoideae as *Cynanchum callialata, Pergularia daemia*, and *Tylophora indicates* (MAHESWARI, 1964). Nonetheless, these layers do not multiply on the anther abaxial surface as in *O. appendiculatum.* The multiseriate tapetum of *O. appendiculatum* might be an autapomorphy, but more studies are needed to prove such assertion. Demarco (2015), who analyzed the secretory structures of Asclepiadeae (Asclepiadoideae), concluded that the glandular tapetum cells secrete a lipid substance that both covers the whole pollinium and is an exine precursor.

The hyaline film that covers the pollinia of *O. appendiculatum*, which, according to Demarco (2015), is composed of sporopollenin secreted by the tapetum, both covers the pollinia and creates compartments isolating pollen grains one from another (VERHOEVEN & VENTER, 2001). *Matelea denticulata* (Vahl) Fontella and E.A. Schwarz present a hyaline film similar to the one found in the species of *Oxypetalum* (SCHILL & JAKEL, 1978). In these species, such presence may be explained by the proximity of subtribes Gonolobinae (*Matelea*) and

Oxypetalinae (*Oxypetalum*) in the "MOOG Clade" (DEMARCO, 2015; LIEDE-SCHUMANN et al., 2005).

The pollinia of Periplocoideae and Secamonoideae are not coated with a hyaline film (Verhoeven & Venter 2001). Tribe Fockeeae (Asclepiadoideae) presents a reduced pollinial wall quite similar to that of species of subfamily Secamonoideae, guaranteeing its maintenance as the most basal among Asclepiadoideae (POTGIETER & ALBERT, 2001).

Our results indicate successive microsporogenesis in O. *appendiculatum*. Species *Calotropis procera* (Aiton) Dryand. and specimens of *Asclepias* L., pertaining to tribe Asclepiadeae, present a microsporogenesis pattern similar to that of *O. appendiculatum,* indicating that such development pattern is shared by the tribe members (CORRY, 1882; DICKO-ZAFIMAHOVA, 1980; ENDRESS & BRUYNS, 2000). Successive microsporogenesis can even be considered a synapomorphy in Secamonoideae and Asclepiadoideae, whose species present true pollinia (ENDRESS & BRUYNS, 2000). The linear tetrads produced by this type of microsporogenesis, as observed in the studied species, are also common in species that present pollen grains agglutinated in pollinia (DEMARCO, 2015; DICKO-ZAFIMAHOVA, 1980).

Oxypetalum appendiculatum presents five pollinaria. Each consists of a pair of hanging pollinia, two caudicles, and a central corpuscle, named retinaculum, with winged lateral expansion. Pollinia orientation in relation to the translator was used to classify Asclepiadoideae into three tribes: (1) Marsdenieae, with erect pollinia; (2) Gonolobeae, with horizontal pollinia; and (3) Asclepiadeae, with hanging pollinia (SWARUPANANDAN et al., 1996). Because of its hanging pollinia, *O. appendiculatum* is inserted in tribe Asclepiadeae.

Size, shape, pollinia orientation, and caudicle and retinaculum positions are mainly used to delimit species (SINHA & MONDAL, 2011; SCHUMANN, 1895; SILVA et al., 2008; FOURNIER, 1885). In *O. appendiculatum*, the winged expansion at retinaculum apex is a character that helps identifying the species (SILVA et al., 2008). Both the retinaculum and the caudicles present a shiny surface and corneous consistency, but retinaculum distinguishes from caudicles through its reddish brown chestnut color and translucent caudicles characterize genus *Oxypetalum* (SILVA et al., 2008).

Finally, the tricellular pollen grains of *O. appendiculatum* are a character shared by most species of Apocynaceae. Nevertheless, bicellular pollen grains have already been reported in *Catharanthus pusillus* L. and *Rauvolfia serpentina (*L.) Benth ex Kurz (LAMBA, 1976). With regard to pollen aperture in family Apocynaceae, the three development stages are reflected in the pollen structure: (1) Rauvolfioideae present colporate or porate pollen; (2) Apocynoideae and Periplocoideae have porate pollen; (3) Secamonoideae and Asclepiadoideae (*Oxypetalum*), which present true pollinia, produce inaperturate pollen (ENDRESS & BRUYNS, 2000). This feature ratifies the presence of *O. appendiculatum* in Asclepiadoideae.

Subsessile stamen and absence of endothecial thickening characterize genus *Oxypetalum* R.Br. The position of O. *appendiculatum* within the group of species derived from this genus is consolidated by the fact that anther filaments do not form a tube. Anther arrangement in three groups, divided according to size and position, has never been reported in the literature. On the other hand, the lack of endothecial thickening is a character shared only by species of tribe Asclepiadeae.

Bilocular anther, biseriate tapetum, successive microsporogenesis, linear tetrads, and inaperturate pollen grains are reported as inherent to the species of subfamily Asclepiadoideae, where *O. appendiculatum* is inserted.

Such features as anther wall development of the dicotyledonous type, secretory tapetum, presence of pollinia, and tricellular pollen grains ratify the taxonomic features of family Apocynaceae.

References

CIVEYREL, L.; ROWE, N. Phylogenetic relationships of Secamonoideae based on the plastid gene matK, morphology, and biomechanics. Annals of the Missouri Botanical Garden. v. 88, p. 583-602, 2001.

COCUCCI, A.A.; MARINO, S.; BARANZELLI, M.; WIEMER, A.P.; SERSIC, A. The buck in the milkweed: evidence of male-male interference among pollinaria on pollinators. New Phytologist. v. 203, p. 280-286, 2014.

CORRY, T.H. On the mode of development of the pollinium in Asclepias cornuti, Decaisne. Transactions of the Linnean Society of London. v. 2, n. 75-84, 1882.

DEMARCO, D. Micromorphology and histochemistry of the laticifers from vegetative organs of asclepiadoideae species (Apocynaceae). Acta Biológica Colombiana. v. 20, p. 57-65, 2015.

DICKO-ZAFIMAHOVA, L. Etude ontogenique de la pollinie de Calotropis procera (Asclepiadaceae): apport de la microscopie photonique. Grana. v. 19, p. 85-98, 1980.

ENDRESS, P.K. Development and evolution of extreme synorganization in angiosperm flowers and diversity: a comparison of Apocynaceae and Orchidaceae. Annals of Botany. v. 117, p. 749–767, 2016.

ENDRESS, M.E.; BRUYNS, P.V. A revised classification of the Apocynaceae s.l. The Botanical Review. v. 66, p. 1-56, 2000.

ENDRESS, M.E.; LIEDE-SCHUMANN, S.; MEVE, U. An updated classification for Apocynaceae. Phytotaxa. v. 159, p. 175-194, 2014.

FOURNIER, E. Asclepiadaceae in Martius. Flora Brasiliensis. v. 6, p. 189-332, 1885.

FYRE, T.C.; BLODGETT, E.B. A contribution to the life history of Apocynum androsamifolium. Botanical Gazette. v. 40, p. 49-53, 1905.

GHIMIRE, B.; GHIMIRE, B.K.; HEO, K. Microsporogenesis in Rauvolfia serpentina (L.) Benth ex Kurz (Apocynaceae): an evidence for dual cytokinesis in microspore mother cells. Journal of Medicinal Plants Research. v. 5, p. 432-438, 2011.

GUIGNARD, L. The egg in Apocynaceae and Asclepiadaceae. Review of Scientific Instruments. v. 55, p. 1-3, 1917.

HARDER, L.D.; AIZEN, M.A. Floral adaptation and diversification under pollen limitation. Proceedings of the Royal Society B: Biological Sciences. v. 365, p. 529-543, 2010.

HARDER, L.D.; JOHNSON, S.D. Function and evolution of aggregated pollen in angiosperms. The International Journal of Plant Sciences. v. 169, p. 59-78, 2008.

HARDER, L.D.; ROUTLEY, M.B. Pollen and ovule fates and reproductive performance by flowering plants. In: LD Harder, SCH Barrett, eds. Ecology and evolution offlowers. Oxford: Oxford Universit Press. 2006.

JUDD, W.S.; CAMPBELL, C.S.; KELLOG, E.A.; STEVENS, P.F.; DONOGHUE, M.J. Sistemática vegetal: um enfoque filogenético. Artmed. 2009.

KARNOVSKY, M.J.A. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. The Journal of Cell Biology. v. 27, p. 137-138, 1965.

KUNZE, H. Morphology of the stamen in the Asclepiadaceae and its systematic relevance. Botanische Jahrbücher für Systematik. v. 118, p. 547-579, 1996.

KUNZE, H. Ontogeny of the translator in the Asclepiadaceae s. str. Plant Systematics and Evolution. v. 193, p. 223-242, 1994.

KOTOVSKI, E.R. Morfoanatomia floral em Allamanda L. (Apocynaceae, Rauvolfioideae). PhD Thesis, State University of Campinas, São Paulo. 2013.

LAMBA, L.C. Microsporogenesis and male gametophyte in Rauvolfia serpentina (L.) Benth. ex Kurz. Agriculture, Biology & Environmental Sciences. v. 45, p. 387-388, 1976.

LIEDE, S. Anther differentiation in the Asclepiadaceae-Asclepiadeae form and function. In: D'ARCY, W. G.; KEANTING, R. C. (Ed.). The anther: form, function and phylogeny. Cambridge: Cambridge University Press. 1996.

LIEDE-SCHUMANN, S.; NIKOLAUS, M.; SILVA, U.C.S.; RAPINI, A.; MANGELSDORFF, R.D.; MEVE, U. Phylogenetics and biogeography of the genus Metastelma (Apocynaceae-Asclepiadoideae-Asclepiadeae: Metastelmatinae). Systematic Botany. v. 39, p. 594-612, 2014.

LIEDE-SCHUMANN, S.; RAPINI, A; GOYDER, D.J.; CHASE, M.W. Phylogenetics of the new world subtribes of Asclepiadeae (Apocynaceae-Asclepiadoideae): Metastelmatinae, Oxypetalinae, and Gonolobinae. Systematic Botany. v. 30, p. 183-194, 2005.

MAHESWARI, D.H. Embryology of Rauvolfia serpentina and Catharanthus roseus. Agriculture, Biology & Environmental Sciences. v. 39, p. 376-377, 1970.

MANNING, J.C. Diversity of endothecial patters in the angiosperms. In: D'ARCY, W.G.; KEATING, R. C. The anther: form, function and phylogeny. Cambridge: Cambridge University Press. 1996.

MEYER, S. Studies in the family Apocynaceae. Journal of Separation Science. v. 1, p. 131-158, 1938.

O'BRIEN, T.P.; FEDER, N.; MCCULLY, M.E. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma. v. 59, p. 368-373, 1964.

PACINI, E. Relationship between tepetum, loculus, and pollen during development. International Journal of Biological Sciences. v. 171, p. 1-11, 2010.

PACINI, E.; FRANCHI, G.G.; HESSE, M. The tapetum: its form, function, and possible phylogeny in Embryophyta. Plant Systematics and Evolution. v. 149, p. 155-185, 1985.

PAIVA, J.G.A.; FANK-DE-CARVALHO, S.M.; MAGALHÃES, M.P.; GRACIANO-RIBEIRO, D. Verniz vitral incolor 500®: uma alternativa de meio de montagem economicamente viável. Acta Botanica Brasilica. v. 20: p. 257-264, 2006.

POTGIETER, K.; ALBERT, V.A. Phylogenetic relationships within Apocynaceae s.l. based on trnL intron and trnL-F spacer sequences and propagule characters. Annals of the Missouri Botanical Garden. v. 88, p. 523-549, 2001.

RAPINI, A.; CHASE, M.W.; GOYDER, D.J.; GRIFFITHS, J. Asclepiadeae classification: evaluating the phylogenetic relationships of New World Asclepiadoideae (Apocynaceae). Taxon. v. 52, p. 33-50, 2003.

RAPINI, A. Taxonomy "under construction": advances in the systematics of Apocynaceae, with emphasis on the Brazilian Asclepiadoideae. Rodriguésia. v. 63, p. 75-88, 2012.

RAU, M.A. Studies in Apocynaceae. The Indian Botanical Society. v. 19, p. 33-44, 1940.

SCHILL, R.; JAKEL, U. Beitrag zur Kenntnis der Asclepiadaceen-Pollinarien. Tropische und Subtropische Pflanzenwelt. v. 22, p. 53-170, 1978.

SCHUMANN, K. Apocynaceae & Asclepiadaceae. In: Die natürlichen Pflanzenfamilien (HGA Engler, KAE Prantl, eds.). Leipzig: Wilhelm Engelmann. 1895.

SILVA, N.M.F; VALENTE, M.DAC.; PEREIRA, J.F.; FILHO, G.M.A.; ANDRADE, L.R. Morfoanatomia de espécies brasileiras de Oxypetalum (Asclepiadoideae-Apocynaceae). Rodriguésia. v. 59, p. 915-948, 2008.

SIMÕES, A.O.; CASTRO, M.M.; KINOSHITA, L.S. Calycine colleters of seven species of Apocynaceae (Apocynoideae) from Brazil. Biological Journal of the Linnean Society. v. 152, p. 387-398, 2006.

SINHA, S.; MONDAL, A.K. The morphological diversification of pollinia of some members of Asclepiadaceae. African Journal of Biotechnology. v. 10, p. 7981-7986, 2011.

SUD, K.C. Contribution to the embryology of Trachelospermum fragrans Hook. F. (Apocynaceae). Plant Science. v. 93, p. 495-501, 1984.

SWARUPANANDAN, K.; MANGALY, J.K.; SONNY, T.K.; KISHOREKUMAR, K.; BASH, C.S. The subfamilial and tribal classification of the family Asclepiadaceae. Biological Journal of the Linnean Society. v. 1220, p. 327-369, 1996.

VALENTE, M.C. A flor de Oxypetalum banskii Roem. et Schult. subsp. banskii: Estudo da anatomia e vascularização (Asclepiadoideae). Rodriguésia. v. 29, p. 161-283, 1977.

VALENTE, M.C.; COSTA, C.G. Estudo anatômico da flor de Marsdenia loniceroides E. Fournier (Asclepiadoideae-Apocynaceae). Rodriguésia. v. 56, p. 51-66, 2005.

VERHOEVEN, R.L.; VENTER, H.J.T. Pollen morphology of the Periplocoideae, Secamonoideae and Asclepiadoideae (Apocynaceae). Annals of the Missouri Botanical Garden. v. 88, p. 569-582, 2001.

VERHOEVEN, R.L.; VENTER, H.J.T. Pollinium structure in Periplocoideae (Apocynaceae). Grana. v. 37, p. 1-14, 1998.

VIEIRA, M.F.; SHEPHERD, G.J. Oxypetalum banksii subsp. banksii: a taxon of Asclepiadaceae with an extra gynoecial compitum. Plant Systematics and Evolution. v. 233, p. 199-206, 2002.